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Simultaneous estimation of Nebivolol and Amlodipine by UV Spectrophotometric method

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Abstract - Nebistar-SA is available for the treatment of stage II hypertension. It contains nebivolol (NV; 5 mg) and amlodipine (AM; 2.5 mg). In the present study, simple, rapid, precise and accurate method for the simultaneous estimation of these drugs have been developed and validated by UV spectrophotometry. The method was validated with respect to its linearity, limit of quantitation (LOQ), limit of detection (LOD), precision and accuracy. In this method, the NV and AM were scanned using methanol as solvent and λ_{max} were found to be 218 nm and 237 nm for NV and AM respectively. For NV (A1 = 0.0182 Cx + 0.0409 Cy) and AM (A2 = 0.0016 Cx + 0.0034 Cy), the equations were developed by Vierodt's method. LOD was found to be 0.086 µg/ml for NV and 0.021 µg/ml for AM. LOQ was 0.262 µg/ml for NV and 0.064 µg/ml for AM. The % RSD for day to day precision was 0.5316 for NV and 0.0056 for AM. The linearity was found to be in the range of 5-50 µg/ml for NV and AM.

Keyword - amlodipine, auxophores, chromophores, nebivolol, uv-vis spectrophotometry,

1. INTRODUCTION

Simultaneous equation method or Vierodt's method

If sample contains two absorbing drugs x and y each of which has absorption maxima at λ_1 and λ_2. It may be possible to determine both drugs by simultaneous equation method. The following criteria may be applied.The information required is:

Let Cx and Cy be the concentrations of x and y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is the sum of

The absorptivities of x at λ_1 and λ_2, ax_1 and ax_2 respectively, the absorptivities of y at λ_1 and λ_2, ay_1 and ay_2 respectively and the absorbance of the diluted sample at λ_1 and λ_2, A_1 and A_2 respectively. The individual absorbances of x and y.

At λ_1 $\quad A_1 = ax_1\ bcx + ay_1\ bcy$

At λ_2 $\quad A_2 = ax_2\ bcx + ay_2\ bcy$

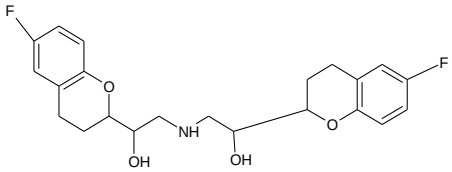
For the measurements in 1cm cells, b = 1.

The criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested that place limits on the relative concentrations of the components of the mixture [1, 2 and 3].

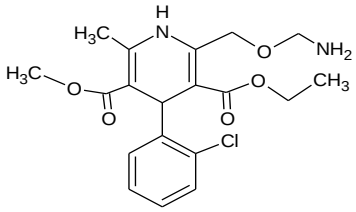
The criteria is that the ratios should be [4]

$$\frac{A_2/A_1}{ax_2/ax_1} \text{ and } \frac{ay_2/ay_1}{A_2/A_1}$$

Nebivolol



Amlodipine



2. MATERIAL AND METHODS

A combination of nebivolol and amlodipine was selected for analysis. These drugs are more effective in combination therapy as compared to monotherapy [5]. The literature reveals that very few spectrophotometric methods are available for the simultaneous estimation of these combinations.Hence, it was thought that a simultaneous estimation for these combinations can be carried out to make the available methods more cost effective. The following steps were undertaken for estimation of drugs in combined dosage form:

Determination of λmax for each drug, preparation of standard curve with drug, development of UV method, application of developed method to marketed product, Nebistar SA and validation and statistical evaluations.

Preparation of calibration curve

Stock solution (s) of nebivolol and amlodipine was suitably diluted to give a concentration of 10 µg/mL and this was scanned in UV range. For nebivolol two absorption maxima were observed at value of 218 nm and 283 nm. The value of 218 nm was chosen for the development of the present procedure. Similarly for amlodipine two absorption maxima were observed at

value of 237 nm and 361 nm. The value of 237 nm was chosen for the development of the present procedure

Working standard solution (100 µg/mL) was made from stock solution(s) by suitably diluting with methanol. Aliquots (0.5, 1.0, 1.5…… 5.0 mL) were taken from this working standard solution and suitably diluted with methanol to give a concentration range of 5 to 50 µg/mL. For nebivolol and amlodipine the absorbance were recorded at 218 nm and 237 nm respectively against a reagent blank and calibration curves was plotted as shown in **Fig. 1, 2** and their overlay spectrum is shown in **Fig. 3**.

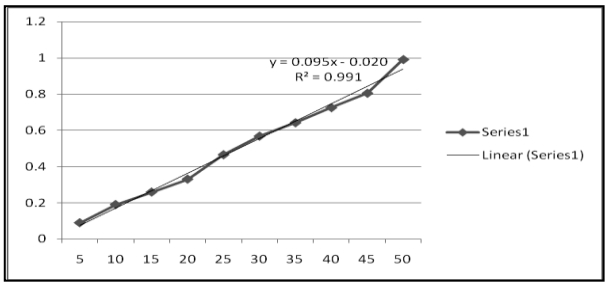


Fig.1. Calibration curve of nebivolol at 218 nm

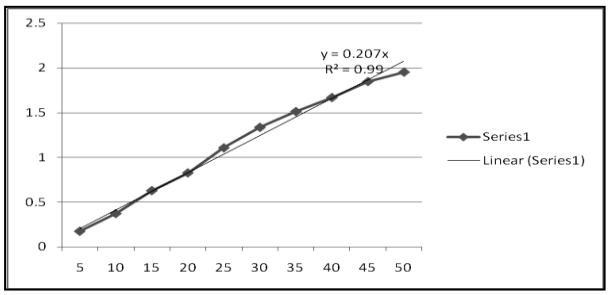


Fig.2. Calibration curve of amlodipine at 237 nm

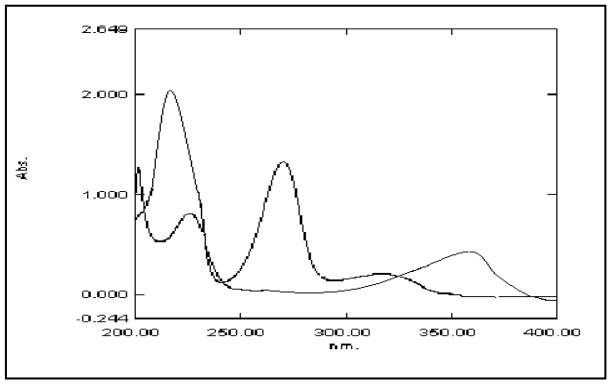


Fig.3. Overlay UV spectra of nebivolol and amlodipine

Optical characteristics

The optical characteristics such as absorption maxima, Beer's law limit, correlation coefficient (r), slope (m), intercept (c), molar absorptivity and Sandell's sensitivity were calculated and the results are incorporated in **Table 1**.

Development of simultaneous equation

For nebivolol and amlodipine by Vierodt's method equations were developed for simultaneous estimation using the following set of equations:

At 218 nm, $A1 = a_{x1}\,bCx + a_{y1}\,bCy$

At 237 nm, $A2 = a_{x2}\,bCx + a_{y2}\,bCy$

Cx and Cy = Concentration of nebivolol and amlodipine respectively in µg /mL.

A1 and A2 = absorbance at 218 nm and 237 nm respectively.

a_{x1} and a_{x2} = absorption coefficient of nebivolol at 218 nm and 237 nm respectively.

a_{y1} and a_{y2} = absorption coefficient of amlodipine at 237 nm and 218 nm respectively.

b = 1 (for measurement in 1 cm. cells).

Table 1: Optical characteristics

Parameters	Values	
	Nebivolol	Amlodipine
λ_{max} (nm)	218	237
Beer's Law Limit (µg/mL)	5-60	5-70
Molar absorptivity (L/mol.cm)*	0.3061×10^4	0.2203×10^4
Sandell's sensitivity (µg cm^{-2}/0.001)*	0.0972	0.1208
Regression equation (y = mx + c)	Y=0.095x-0.02	y = 0.207x
Slope (m)	0.095	0.207
Intercept (c)	0.02	0.0
Correlation coefficient (r)	0.991	0.999

Estimation from tablets

Tablet formulation Nebistar–SA has the composition of 5 mg of nebivolol and 2.5 mg of amlodipine was chosen for analysis. Tablets were procured from the local market and the average weight was determined. The tablets were powdered and powder equivalent to 100 mg nebivolol and 50 mg amlodipine were taken in 100 mL of conical flasks separately. These were extracted with methanol (4x20 mL) and filtrate was taken in 100 mL volumetric flasks and the volumes were made up to 100 mL with methanol. Aliquots of a definite concentration were further suitably diluted to give the concentration in the range of 5-50 µg/mL. The drug content in the tablets was calculated [6]. The experiments were repeated six times to check its reproducibility and the results are shown in Table 2 and 3.

Method Validation

Accuracy

Accuracy was performed by standard addition method (recovery study) using three variants. Three repetitions

were done with each variant (addition). Stock working standard solutions of each nebivolol and amlodipine (100 µg/mL) were prepared. A solution of tablet formulation equivalent to 100 µg/mL nebivolol and 50 µg/mL of a-mlodipine respectively was prepared.

Linearity

By preparing different dilutions ranging from 5-50 µg/mL of both the drugs and detected under UV light. The concentration of nebivolol at 218 nm and amlodipine at 237 nm was found linear range of 5-50 µg/mL.

Precision

It is a measure of degree of repeatability or reproducibility under normal conditions.

Limit of Detection (LOD)

Based on the standard deviation of the response and slope detection limit may expressed as:

$$LOD = 3.3\ \sigma/s$$

Where, σ = Standard deviation of the response.

S = Slope of the calibration curve.

Limit of Quantitation (LOQ)

Based on the standard deviation of the response and the slope quantitation limit may be expressed as:

$$LOQ = 10\ \sigma/S$$

3. Results And Discussion

The present work comprised of development of an analytical method for the simultaneous estimation of nebivolol and amlodipine by UV spectrophotometry, as well as, validation of the developed method. The commercially available tablet dosage forms selected for the estimation was Nabistar-SA of Lupin Ltd which contained nebivolol-5 mg and amlodipine-2.5 mg.

In UV Spectrophotometry, the Vierodt's method was followed for the analysis. The solvent methanol was selected on the basis of solubility and stability. The λmax was determined for each drug nebivolol and amlodipine which were found as 218 and 237 nm respectively. The calibration curve was plotted between concentration and AUC measured at the selected wavelength of 218 nm and 237 nm. The concentration of drugs in the tablet was found by using slope and intercept of linearity curve. Validation challenges showed that the methods show reproducibility when carried out by different persons, in the same or different laboratories using different reagents etc.

Further the estimation of nebivolol and amlodipine was carried out on marketed formulations Nebistar SA by using prepared standard curves.

Developed equations for simultaneous estimation by UV spectrophotometry

Amlodipine, $A_1 = 0.0182Cx + 0.0409Cy$

Nebivolol, $A_2 = 0.0016Cx + 0.0034Cy$

Where, A= Absorbance, C= Concentration

Table 2: Estimation of drugs from nebistar-SA tablet

S. No.	Absorbance		Concentration (µg/mL)		Amount found per tablet (mg)	
	218 nm	237 nm	NV (Cx)	AM (C_Y)	X	Y
1	0.3869	0.0330	10.0025	05.0086	5.0012	2.5043
2	0.3870	0.0330	10.0042	05.0093	5.0021	2.5046
3	0.3863	0.0329	09.9968	04.9968	4.9984	2.4984
4	0.3864	0.0329	09.9984	04.9984	4.9992	2.4992
5	0.3869	0.0332	10.0024	05.0087	5.0012	2.5043
6	0.3865	0.0330	10.0037	04.9986	5.0019	2.4993

Table 3: Statistical analysis of Nebistar–SA tablets

Parameters	Nebivolol	Amlodipine
Standard deviation	0.00249	0.00134
Coefficientof variation	0.000498	0.000536
Standard error of mean	0.001017	0.000547
Percentage range of error (within 95% confidence limits)	0.001993	0.001072
Label claim* (mg/tab)	5.0	2.5
Amount found* (mg/tab)	4.998	2.50015
SD*	0.00249	0.00134
%RSD*	0.04979	0.05356
SEM*	0.00102	0.00056

*Average of six determinations.

Validation of UV spectrophotometric method

The results of validation are summarized in Table 4.

Table 4: Validation data for the developed UV spectroscopic method

Validation Parameters	Nebistar SA	
	NVL	AMLO
Linearity (r^2)	0.9997	0.9996
Precision (%SD)		
Analyst variation	0.0002	0.0001
Inter day Variation	0.0260	0.0001
Accuracy (%SD)	0.4900	0.3700
Tablet analysis		
%found	49.98	25.01
LOD	0.090	0.020
LOQ	0.260	0.060

4. Conclusion

The UV spectrophotometric method developed is simple, precise, rapid, selective and economical for the simultaneous estimation of nebivolol and amlodipine in solid dosage form. It can also be used for the analysis of these drugs in biological fluids and in quality control laboratories.

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Dr. Asmita Gajbhiye has about 15 years experience of research and teaching experience at both UG and PG levels.She is a well renowned scientist who has published more then 25 papers in journals of international and national repute and presented more then 50 papers in the various conferences/ seminars and symposia at national and international level. She has successfully completed the various research projects at PG and Ph. D. level. She has also received the best presentation awards at national level. Her research projects have been appreciated at international level during presentation of research papers. She has delivered invited lectures and chaired many sessions in several National and International conferences and symposia in India and abroad. Presently, she is working as Associate Professor in Department of Pharmaceutical Sciences, Dr. H.S. Gour Central University, Sagar, MP.